

# Heat Production by Ruminal Bacteria in Continuous Culture and Its Relationship to Maintenance Energy

JAMES B. RUSSELL

Agricultural Research Service, U.S. Department of Agriculture, and Department of Animal Science, Cornell University, Ithaca, New York 14853

Received 23 April 1986/Accepted 13 August 1986

*Selenomonas ruminantium* HD4 and *Bacteroides ruminicola* B<sub>14</sub> were grown in continuous culture with glucose as the energy source, and heat production was measured continuously with a microcalorimeter. Because the bacteria were grown under steady-state conditions, it was possible to calculate complete energy balances for substrate utilization and product formation (cells, fermentation acids, and heat). As the dilution rate increased from 0.04 to 0.60 per h, the heat of fermentation declined from 19 to 2% and from 34 to 8% for *S. ruminantium* and *B. ruminicola*, respectively. At slow dilution rates the specific rate of heat production remained relatively constant (135 mW/g [dry weight] or 190 mW/g of protein for *S. ruminantium* and 247 mW/g [dry weight] or 467 mW/g of protein for *B. ruminicola*). Since the heat due to growth-related functions was small compared to maintenance expenditures, total heat production provided a reasonable estimate of maintenance under glucose-limiting conditions. As the dilution rate was increased, glucose eventually accumulated in the chemostat vessel and the specific rates of heat production increased more than twofold. Pulses of glucose added to glucose-limited cultures (0.167 per h) caused an immediate doubling of heat production and little increase in cell protein. These experiments indicate that bacterial maintenance energy is not necessarily a constant and that energy source accumulation was associated with an increase in heat production.

Biological growth depends on the transfer of energy from catabolic to anabolic processes, but the conversion is never complete. At each step, energy is dissipated into the environment as heat. When heat production increases, there is less energy to do the chemical work involved in growth, and efficiency decreases. Calorimetric methods of measuring heat production provide a continuous and nondisturbing way to monitor metabolism, and sensitivity is no longer a problem (5, 8-10, 21, 25). In spite of these apparent advantages, there have been few, if any, applications of microcalorimetry to the study of microbial growth efficiency.

Continuous-culture studies have indicated that cell yields were generally lower at slow growth rates, and the idea was introduced that energy could be used for functions not directly related to growth (11). These nongrowth functions were designated maintenance energy. Since maintenance is the amount of energy that cannot be used for growth, it is apparent that maintenance energy must contribute to heat production.

In the 1960s, Marr et al. (23) and Pirt (27) described maintenance energy by using double-reciprocal plots of growth rate and cell mass or yield. Transformation of the data as a reciprocal, however, introduced a statistical bias. Plots using the specific rate of substrate utilization versus growth rate tended to provide a more even distribution of points, and deviations from linearity were more easily recognized (26, 43, 47). In all of these derivations, maintenance was assumed to be a time-dependent function that was proportional to cell mass and independent of growth rate. Energy-sufficient continuous cultures, in many cases, had disproportionately lower cell yields (26, 43, 45, 46), and this disparity, although still a subject of controversy, suggested that maintenance might have a growth rate-dependent component (28).

When growth rate is high, maintenance makes up a relatively small proportion of total energy use. However, as

the growth rate decreases, maintenance becomes more significant. All of the energy is used for maintenance when growth ceases. Within the rumen, the concentration of soluble energy sources is usually very low, cell density is high, and average growth rate is low, so that maintenance exerts a significant impact on cell yield (32-34). Because microbes are an important source of amino acids for the animal, there is an economic motivation for increasing microbial growth yields in the rumen (16).

The following series of experiments measured the heat production of two predominant ruminal bacteria, *Selenomonas ruminantium* and *Bacteroides ruminicola*, grown in continuous culture. The results indicated that (i) specific heat production remained relatively constant when glucose was limiting, (ii) specific heat production rates increased markedly when the dilution rate approached the maximum growth rate and glucose accumulated, and (iii) the addition of small amounts of glucose at slow dilution rates caused immediate increases in heat production, even though there was little increase in the amount cell protein or dry weight.

## MATERIALS AND METHODS

**Cell growth and sampling.** The basal medium contained 292 mg of K<sub>2</sub>HPO<sub>4</sub>, 292 mg of KH<sub>2</sub>PO<sub>4</sub>, 480 mg of (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 480 mg of NaCl, 100 mg of MgSO<sub>4</sub> · 7H<sub>2</sub>O, 64 mg of CaCl<sub>2</sub> · 2H<sub>2</sub>O, 4,000 mg of Na<sub>2</sub>CO<sub>3</sub>, 100 mg of Trypticase (BBL Microbiology Systems, Cockeysville, Md.), 100 mg of yeast extract, 600 mg of cysteine hydrochloride, and 5.5 mmol of glucose per liter, a micromineral solution (37), and vitamins (5). Medium for *S. ruminantium* was supplemented with biotin (4 µg/ml) (39) and 1 mM valerate (37), and the medium for *B. ruminicola* received 1.0 mM isobutyrate, isovalerate, 2-methylbutyrate, and valerate (30).

Cells were grown under strictly anaerobic conditions as described by Hungate (15), and the methods for continuous culture have been described previously (32). Optical density

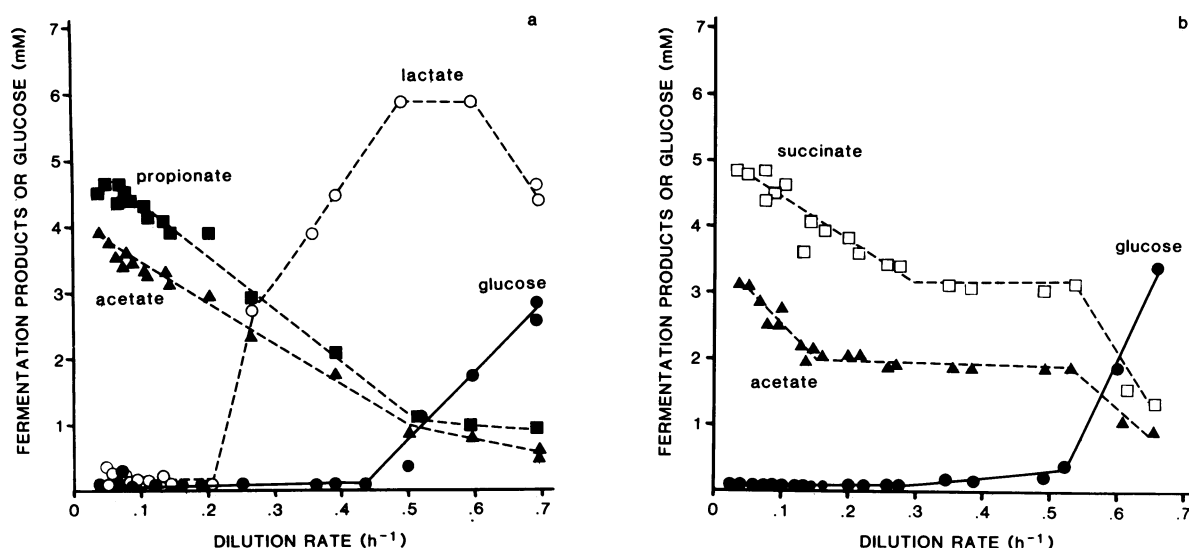


FIG. 1. Fermentation of glucose by *S. ruminantium* (a) and *B. ruminicola* (b) in continuous culture (glucose, ●; acetate, ▲; propionate, ■; lactate, ○; and succinate, □).

(Gilford 260 spectrophotometer, 600 nm, cuvettes of 1-cm light path) and heat production (described later) were used as an index of steady state. A steady state was always obtained after four culture volumes flowed through the 360-ml vessel. After each sampling the chemostat was reinoculated with 250 ml of cells grown in batch culture. Incubation temperatures was 39°C. Cells were separated from the culture supernatant by passing the culture fluid (5 ml) through a membrane filter (0.65  $\mu$ m). Cells from the remaining 355 ml of culture were harvested by centrifugation (8,000  $\times$  g, 0°C, 15 min) and washed in distilled water. Preliminary experiments showed that distilled-water washing gave the same amount of cell protein as washing in 0.9% NaCl. The cell-free supernatant and cell samples were stored at -15°C until analysis.

**Analysis.** Volatile fatty acids, lactate, and succinate in cell-free supernatant samples were analyzed by high-pressure liquid chromatography with a Beckman 334 liquid chromatograph, a model 156 refractive index detector, and a Bio-Rad HPX-87H organic acid column. The sample size was 50  $\mu$ l, the eluant was 0.013 N H<sub>2</sub>SO<sub>4</sub>, and the column temperature was 50°C. Ammonia was measured by the method of Chaney and Marbach (4), and the glucose was analyzed by an enzymatic method with hexokinase and glucose-6-phosphate dehydrogenase (31).

Protein from NaOH-hydrolyzed cells (0.2 N, 100°C, 15 min) was assayed by the method of Lowry et al. (20). Cell nucleic acids were extracted with hot 0.5 N perchloric acid. DNA and RNA in the supernatants were determined by the diphenylamine and orcinol procedures of Burton (3) and Schneider (40), respectively. Cell carbohydrate was assayed by the anthrone method with glucose as the standard (1). Preliminary experiments (data not shown) indicated that the anthrone method had little interference from RNA and reacted more completely with bacterial cell wall carbohydrates than an HCl-phenol procedure (19). Cells were dried at 105°C for 2 h and weighed on aluminum pans to determine cell dry weight.

Bacterial heat production was measured with an LKB model 2277 bioactivity monitor that was equipped with semiconducting Peltier elements as thermopiles and gold flow cells. This machine was calibrated with an internal

electric heat source and gave very stable digital and strip chart readouts. The baseline noise was approximately 0.1  $\mu$ W with a flow rate of 0; however, a small amount of surging due to peristaltic pumps increased the noise to approximately 0.5  $\mu$ W in the flow mode. Bacterial cells and medium were pumped from the continuous-culture vessel through Teflon tubes at a flow rate of 40 ml/h. The flow cell temperature was set at 39.00°C, and total transit time from the continuous-culture vessel to the flow cell and back to the culture vessel was approximately 4 min. LKB indicated that the effective volume of the flow cell was 0.678 ml at a flow rate of 40 ml/h, and a variation of as much as 10 ml/h had less than 1% effect on effective volume.

Bacterial cells were dried (105°C) to a constant weight in platinum fuel capsules and burned in a Parr model 1241 calorimeter that was equipped with a model 1107 semimicro-oxygen bomb. The calorimeter was calibrated with calorific-grade benzoic acid, and it was assumed that 1.000 g would yield 6,323 cal (26,455 J). The *S. ruminantium* cells contained an average of 4.61 cal/mg (dry weight), and the *B. ruminicola* cells had 4.78 cal/mg. The dilution rate did not have a significant impact on the caloric content of the cells.

Energy balances were determined by multiplying concentrations of glucose, Trypticase, fermentation acids, and cells by the respective energy value and dilution rate: mmol/liter  $\times$  cal/mmol  $\times$  1/h = cal/liter per h. Glucose, acetate, propionate, lactate, and succinate were given values of 673, 209, 367, 326, and 357 cal/mmol, respectively (12). The manufacturer indicated that Trypticase was 10% ash, and organic matter was assumed to have the same energy content as casein, 5.86 cal/mg (22). The energy content of the cells was determined directly (see above). Assuming that 1.10 cal/h is equivalent to 1.16 mW, then cal/h per liter  $\times$  1.16 mW/cal per h = mW/liter. The rate of heat production was calculated from the quotient of the bioactivity monitor readout and the effective volume as  $\mu$ W/0.678 ml =  $\mu$ W/ml = mW/liter.

**Statistics.** The linearity of relationships was ascertained by linear regression, where  $a$  was the slope term,  $b$  was the intercept, and  $r$  was the correlation coefficient. Statistical variation was estimated by the standard error of the mean (41).

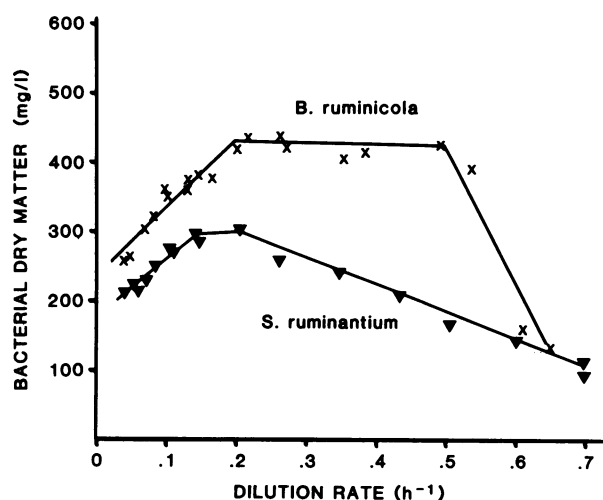


FIG. 2. Effect of dilution rate on production of bacterial dry matter in continuous culture.

## RESULTS

**Fermentation products.** When *S. ruminantium* HD4 was grown in continuous culture with glucose, acetate and propionate were the predominant products as long as dilution rate was less than 0.2 per h (Fig. 1a). Little lactate was produced at dilution rates below 0.2 per h, but at faster dilution rates there was a large increase in lactate and a decrease in acetate and propionate. As dilution rate increased from 0.43 to 0.70 per h, glucose accumulated in the vessel, and this increase could be explained by the substrate affinity and maximum growth rate of the organism ( $\mu_{\max}$ ). A Lineweaver-Burk plot indicated that the affinity constant,  $K_s$ , was 0.153 mM, and  $\mu_{\max}$  was 0.73 per h. *B. ruminicola* B<sub>14</sub> produced succinate and acetate in continuous culture, and the ratio of succinate to acetate was approximately 1.6 regardless of the dilution rate (Fig. 1b). Glucose started to accumulate in the culture vessel at a dilution rate of 0.35 per h, while the estimates of  $K_s$  and  $\mu_{\max}$  were 0.138 mM and 0.68 per h, respectively.

Bacterial dry matter reached a maximum at intermediate dilution rates and declined when the dilution rate was very slow or so fast that glucose accumulated in the culture vessel (Fig. 1 and 2). *S. ruminantium* switched to lactate fermentation at 0.15 per h, and this change accentuated the decrease in bacterial dry matter. As the dilution rate increased, the protein content (as a percentage of total dry weight) of the *S. ruminantium* cells decreased, while RNA and, to a smaller extent, carbohydrate increased (Fig. 3a). DNA ranged from 0.5 to 1.2% of the dry weight and was not affected by the dilution rate. The RNA content of *B. ruminicola* was not influenced significantly by the dilution rate, but there was a modest decrease in protein and an increase in carbohydrate at faster dilution rates (Fig. 3b). The recovery of carbon, electrons, and cell dry matter is shown in Table 1.

**Glucose consumption rates.** When the specific rate of glucose consumption was plotted against the dilution rate ( $n = 15$ ), the intercept, and indicator of maintenance, was 0.192 mmol of glucose/g (dry weight) per h for *S. ruminantium* (Fig. 4a). At dilution rates greater than 0.43, the plot deviated from linearity, and if these points were included the intercept was negative ( $-0.099$  mmol of glucose/h per g [dry weight]). The maintenance coefficient of the *B. ruminicola* culture was 0.240 mmol of glucose/g (dry weight) per h (Fig. 4b).

**Heat production rates.** When heat production was measured continuously with a flow calorimeter, the specific rate of heat production remained relatively constant as long as glucose was completely depleted. At dilution rates less than 0.43, *S. ruminantium* produced 135 mW/g (dry weight) (Fig. 5a). Similar trends were observed with *B. ruminicola* (Fig. 5b). At dilution rates less than 0.27 per h, glucose was not detected in the culture vessel, and the specific rate of heat production was 247 mW/g. When the specific heat production rate was expressed in terms of cell protein instead of dry weight, there was once again little change in the rate until glucose accumulated.

**Pulses of glucose.** The effect of glucose on heat production was examined further with pulse experiments (Fig. 6). In each case, the organisms were grown under glucose-limiting, steady-state conditions at a dilution rate of 0.167 per h. At time zero, 1.0 mM glucose was injected into the culture

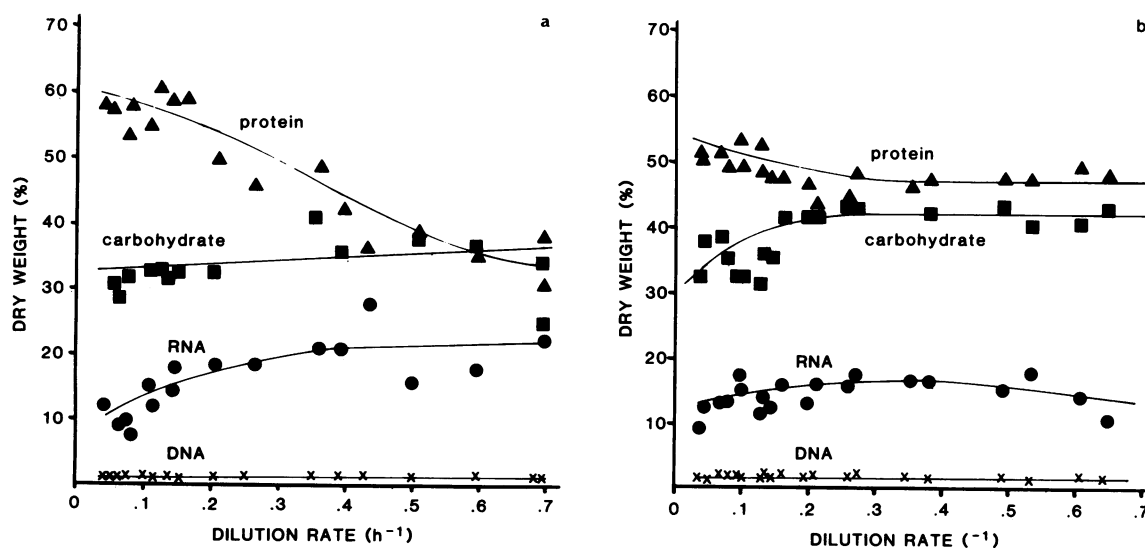


FIG. 3. Composition of *S. ruminantium* (a) and *B. ruminicola* (b) cells grown in continuous culture.

TABLE 1. Fermentation balances<sup>a</sup>

Recovery	% of total $\pm$ SEM	
	<i>S. ruminantium</i>	<i>B. ruminicola</i>
Carbon <sup>b</sup>	98.7 $\pm$ 2.0	94.4 $\pm$ 1.0
Electrons <sup>c</sup>	90.4 $\pm$ 0.9	97.7 $\pm$ 1.2
Cells <sup>d</sup>	104.0 $\pm$ 1.0	96.0 $\pm$ 3.4
Energy <sup>e</sup>	102.7 $\pm$ 2.2	104.6 $\pm$ 2.4

<sup>a</sup> Averaged across 19 steady states  $\pm$  standard error of the mean.<sup>b</sup> Assumes that cells are 50% carbon.<sup>c</sup> Included cells and assumed that cells have an oxidation reduction value of 1.90 (17).<sup>d</sup> Recovery of cells as protein, carbohydrate, RNA, and DNA.<sup>e</sup> After conversion of glucose, Trypticase, yeast extract, fermentation acids, cells, and heat to milliwatts per liter.

vessel, and there was an immediate increase in heat production. The rate of heat production remained elevated until the glucose was completely fermented and then returned to the initial value. *S. ruminantium* produced lactate during this time, while *B. ruminicola* produced succinate and acetate. There was little increase in protein (Fig. 6) or dry weight (data not shown) after glucose injection.

**Energy balances.** Because the bacteria were grown under steady-state conditions, it was possible to calculate a balance between the rates of substrate utilization and product formation. Energy rates (power) were calculated by multiplying concentration, the heat of combustion, and the dilution rate. Heat of combustion values for bacterial cells were not available, and these numbers were determined directly with a bomb calorimeter. Calculation of a complete energy rate balance was confounded by a small amount of Trypticase and yeast extract in the medium, which was not measured. Assuming that *B. ruminicola* and *S. ruminantium* used 40 and 20% of the Trypticase, respectively (6), and all of the yeast extract, the total energy rate balances were 102.7  $\pm$  2.2 and 104.6  $\pm$  2.4% for *S. ruminantium* and *B. ruminicola*, respectively (Table 1).

The distribution of energy in the products as a function of dilution rate is shown in Fig. 7. With *S. ruminantium* there

was an inflection at a dilution rate of approximately 0.15 per h, and this inflection coincided with changes in products. At dilution rates from 0.04 to 0.15 per h, acetate and propionate were the primary products (Fig. 1a). When the dilution rate was greater than 0.15 per h, lactate became the primary product, the number of cells declined, and more energy was found in the fermentation products. *B. ruminicola* did not change fermentation products (Fig. 1b), and neither cell nor fermentation acid plots contained an inflection. As the dilution rate increased, heat production declined (Fig. 7). In the case of *S. ruminantium* heat ranged from 19 to 2%, while the *B. ruminicola* value ranged from 34 to 8%.

## DISCUSSION

With the advent of continuous-culture techniques in the late 1940s it became apparent that the production of bacterial cell mass was related to bacterial growth rate, and the concept of maintenance energy was introduced to explain these differences in yield (11). Maintenance has not been described precisely but functions such as ion balance across the cell membrane, protein turnover, and motility are undoubtedly important. In the mathematical derivation of Marr et al. (23), maintenance is defined as a fractional rate ( $a$ ) which is used to predict the number of additional cells ( $ax$ ) that would be obtained if there were no maintenance requirement. The derivation of Pirt (27) likewise relates maintenance to the differences between the theoretical maximum growth yield ( $Y_G$ , no maintenance) and the observed yield ( $Y$ ).

Since maintenance has generally been defined in terms of the reciprocal of yield and time (micromoles of glucose per gram of bacteria per hour), it is apparent that any factor affecting yield will affect the estimate of maintenance. When Pirt plotted yield data for *S. ruminantium*, the relationship was not linear, and Pirt concluded that "it seems doubtful whether the rapid fall in yield of the selenomonad with decreasing growth rate can be attributed to the maintenance energy requirement" (27). Subsequently, Scheifinger et al. showed that *S. ruminantium* changes from acetate and propionate fermentation to lactate at a dilution rate of

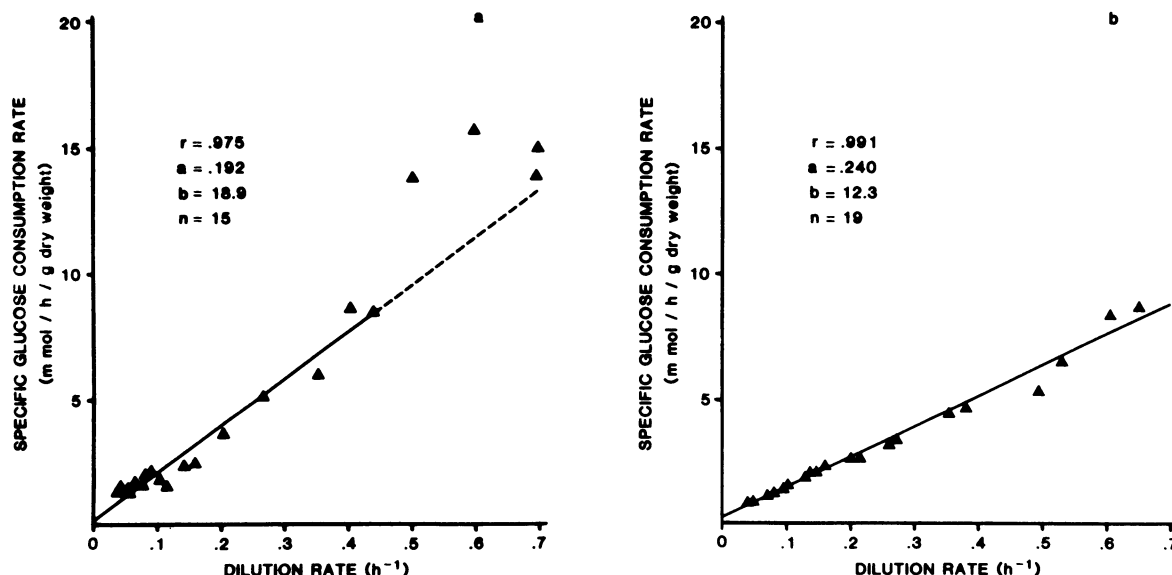


FIG. 4. Specific glucose consumption rate (micromoles of glucose per hour per gram [dry weight]) of *S. ruminantium* (a) and *B. ruminicola* (b) in continuous culture.  $r$ ,  $a$ ,  $b$ , and  $n$  are defined in the text.

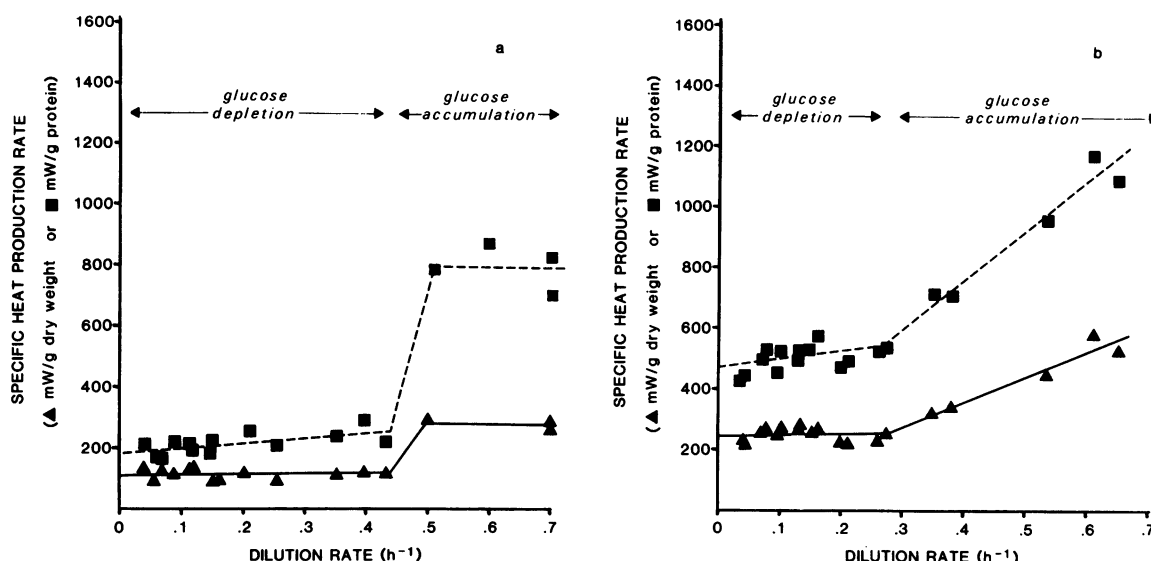


FIG. 5. Specific heat production rate of *S. ruminantium* (a) and *B. ruminicola* (b) in continuous culture. During glucose depletion, the *S. ruminantium* plots had an intercept of 135 and 190, slopes of 119 and  $-72$ , and correlation coefficients of 0.47 and 0.13 for protein and dry weight, respectively. The *B. ruminicola* plots had intercepts of 467 and 247, slopes of 262 and  $-11$ , and correlation coefficients of 0.45 and 0.04, respectively.

approximately 0.2 per h (38). Since the switch in fermentation products affected the amount of ATP that would be available for growth, it is not surprising that the estimate of maintenance was not constant. Changes in fermentation

products and yield values make it difficult (if not impossible) to measure maintenance in this organism, and there is considerable deviation between the present estimate of 0.192 mmol of glucose per g (dry weight) per h (Fig. 4a) and a previous value of 0.122 (33). With *B. ruminicola* B<sub>14</sub> the estimate of maintenance was not confounded by changes in fermentation products, and the coefficient of 0.240 mmol of glucose per g (dry weight) per h (Fig. 4b) was in reasonable agreement with an earlier value of 0.283 (30).

Theoretical calculations by Stouthamer indicate that approximately 3.2 times as much energy is needed to form a gram of cell protein as of polysaccharide (42). An accumulation of polysaccharide at rapid growth rates would tend to increase the yield values, increase the slope of a Pirt plot, and increase the estimate of maintenance. The cell composition of *B. ruminicola* was not affected greatly by the dilution rate (Fig. 3b), but the protein content of *S. ruminantium* was noticeably lower at rapid dilution rates (Fig. 3a). From a more practical point of view, yields of ruminal bacteria should be expressed in terms of protein because amino acids are the most valuable component of ruminal microbial biomass. Studies have indicated that the amino acid supply can limit ruminant animal production (13, 36).

Experiments by Neijssel and Tempest showed that nitrogen-, sulfur-, and phosphate-limited cultures consumed energy sources at a rapid rate even though the rate of biomass formation was slow (26). These differences in yield led Neijssel and Tempest (26) to question the mathematical derivations originally put forth by Marr et al. (23) and Pirt (27). They indicated that maintenance might not be independent of growth rate and that it might be a "simple function of growth rate" (26). Since energy source catabolism is usually tightly coupled to ATP formation, they and other workers suggested that bacteria must have mechanisms to hydrolyze ATP when an energy source is in excess (7, 28, 43, 47). The terms overflow metabolism, slip reactions, energy-spilling reactions, and futile cycles have all been used to explain this

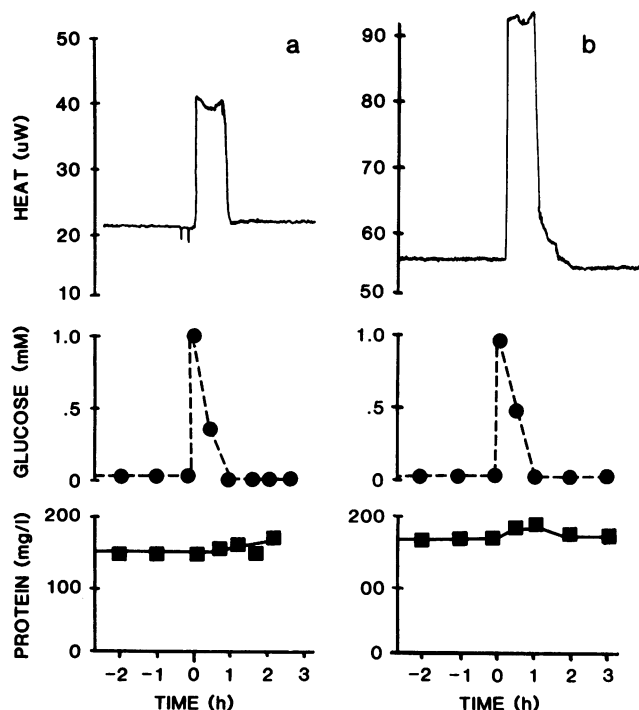


FIG. 6. Effect of a pulse dose of glucose on the heat production of *S. ruminantium* (a) and *B. ruminicola* (b). The flow cell had an effective volume of 0.678 ml, 1.0 mmol of glucose per liter was injected into the chemostat (0.167 per h) at time zero, and the changes in heat, glucose (●), and cell protein (■) were monitored.

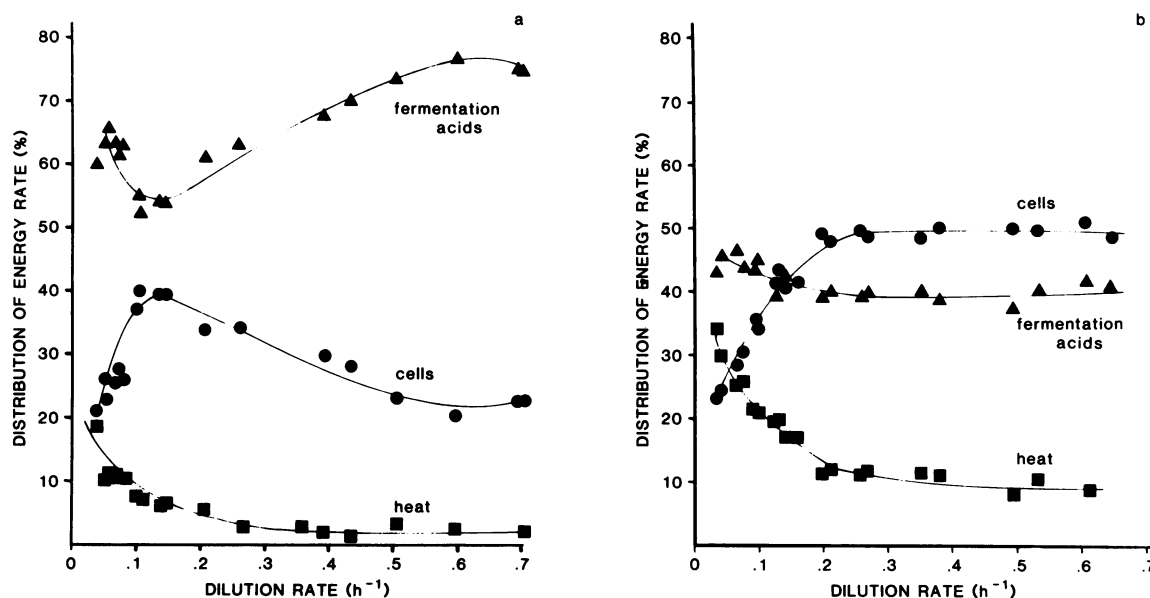


FIG. 7. Distribution of energy in the fermentation of glucose by *S. ruminantium* (a) and *B. ruminicola* (b) grown in continuous culture.

“uncoupling” of catabolism and anabolism, but there has been little definitive information about cellular mechanisms.

Since bacterial yield can be influenced by changes in the end products of metabolism, the composition of cells, and the type of nutrient limiting growth, the indiscriminate use of yields to estimate maintenance could lead to misunderstanding. Heat is the direct product of maintenance functions, and it seemed that measurements of heat production might give a more straightforward interpretation of maintenance energy. Growth would also give rise to heat; however, the amount of heat resulting from growth should be reasonably constant as long as the data are expressed per unit of cell protein. Heat due to growth would be directly proportional to the amount of cell protein and not necessarily a simple function of growth rate.

If one assumes that energy sources are used for growth and maintenance and that maintenance is independent of growth rate, the specific rate of energy utilization ( $q$ ) is given by the linear equation (28)  $q = (\mu/Y_G) + m$ , where  $q$  is the units of energy per hour per gram of cell material,  $\mu$  is the growth rate,  $Y_G$  is the theoretical maximum growth yield (grams of cells per energy), and  $m$  is the maintenance coefficient (energy per gram of cells per hour).

When the ruminal bacteria were grown under glucose-limiting conditions, the specific rate of heat production ( $q$ ) remained relatively constant (Fig. 5). Because the slope of the plot  $1/Y_G$  was small or negligible, most of the heat resulted from  $m$ , the intercept, and little of the heat was associated with growth. But wouldn't growth-related functions give rise to heat? Theoretical calculations by Stouthamer indicated that 0.04 mol of ATP was required to synthesize 1 g of bacterial protein (43). Assuming 7 kcal/mol of ATP and 1.16 mW/cal per h, 325 mW of heat per h would result from the synthesis of 1 g of bacteria. This calculated value is somewhat higher than  $1/Y_G$  values for *B. ruminicola* and *S. ruminantium*, 262 and 119 mW/h per gram of bacterial protein, respectively (Fig. 5). It should be emphasized, however, that these slopes were generally low, had correlation coefficients less than 0.5, and did not account for ATP use in the synthesis of other cell material besides protein.

When specific heat production was expressed per unit of dry weight, the slopes were slightly negative rather than positive. This anomaly was most likely due to changes in cell composition (Fig. 3), which would tend to increase yield at rapid dilution rates and thus decrease the slope or  $1/Y_G$  term (see above).

Since the heat of growth (slope) was insignificant relative to maintenance energy (intercept), total heat production provided a reasonable estimate of maintenance in these two organisms provided that glucose was limiting (Fig. 5). Because most other bacteria have maintenance energy coefficients that are as high as or higher than those of these two species (27, 43, 45, 46, 47, 49) and since the amount of ATP (and hence heat) required to synthesize macromolecules is probably reasonably constant across bacterial species (42), the use of total heat production to estimate maintenance is likely to be valid in other cases as well.

Since energy from glucose can be retained in bacterial biomass, found in end products (if oxidation is incomplete), or dissipated as the heat of maintenance and growth, the use of specific glucose consumption rates to estimate maintenance is analogous to estimating the weight of a man from the difference between a railroad locomotive with and without the man. The slope, or  $1/Y_G$ , is always large if the ordinate is expressed as the specific rate of glucose consumption. A small  $m$  term relative to  $1/Y_G$  means that the estimate of  $m$  is not terribly precise even though the data appear to fall on a straight line. In anaerobic systems the situation is even worse because yields are generally low and this means that  $1/Y_G$  is even larger relative to  $m$ .

Since heat is ultimately derived from the difference in free energy of catabolism and anabolism, the specific rate of heat production should be related to the specific rate of glucose consumption. During glucose limitation, *S. ruminantium* and *B. ruminicola* had  $m$  values of 135 and 247 mW of heat per g of dry matter, respectively (Fig. 5). Assuming 1.16 mW/cal per h, 673 cal/mmol of glucose, and a 55% loss of enthalpy in the conversion of glucose to either acetate and propionate or acetate and succinate, the glucose consumption rates would have been 0.307 and 0.575 mmol/h per g of dry matter for *S.*

*ruminantium* and *B. ruminicola*, respectively. These latter values are significantly higher than the  $m$  terms derived in Fig. 4. However, given the problems in estimating maintenance energy from glucose consumption (see above), the agreement seems reasonable.

When glucose accumulated in the chemostat vessel, there was a marked increase in the specific rate of heat production, especially if the data were expressed in terms of cell protein (Fig. 5). Non-steady-state (pulse) experiments likewise indicated that addition of 1 mM glucose caused a dramatic increase in heat production even though there was little change in either bacterial protein or dry matter (Fig. 6). These results showed that fermentation and growth were not tightly coupled processes and that energy could be dissipated in the form of heat. A teleological yet important question is what advantage organisms get from the increased heat production. Westerhoff theorized that microbial growth is analogous to free-energy ethalpy changes in nonequilibrium thermodynamics: "some thermodynamic efficiency may be sacrificed to make the process run faster" (48). The waste of energy source as heat may also be a means of denying substrate to competing organisms.

The mechanisms involved in the heat or energy spilling need to be elaborated further. According to the chemiosmotic hypothesis of Mitchell (24), most bacteria translocate protons outward through the membrane to establish a proton gradient ( $\Delta pH$ ). Translocation of the protons leaves the interior more negative than the outside, and this difference creates an electrical gradient ( $\Delta\psi$ ). Since the free energy of ATP hydrolysis or electron transport systems (less important in most strict anaerobes) is needed to create the proton motive force (2, 18, 35), dissipation of the proton motive force could provide a mechanism for ATP hydrolysis and heat production. A proton leak or an increase in motility could decrease the proton motive force. *S. ruminantium* is a motile organism, and motility is greater when glucose accumulates. Tempest noted that fast-growing bacteria contain within the cell greater concentrations of potassium, magnesium, and ammonium ion (44). It is conceivable that the ruminal bacteria could have used the proton motive force to transport more of these ions (29) in preparation for an eventual increase in growth rate.

#### ACKNOWLEDGMENT

This research was supported by the United States Dairy Forage Research Center, Madison, Wis.

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